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STUDIES OF THE EGGS OF *MACROBIOTUS* CF. *PSEUDOHUFELANDI* (TARDIGRADA) FROM WHEAT FIELDS IN SOUTH AUSTRALIA

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Summary

BIRD, A. F. & MCCLURE, S. G. (1997) Studies of the eggs of *Macrobiotus* cf. *pseudohufelandi* (Tardigrada) from wheat fields in South Australia. *Trans. R. Soc. S. Aust.* **121**(2), 51-57, 30 May, 1997.

The tardigrade, *Macrobiotus* cf. *pseudohufelandi*, and its eggs were isolated from soil from a wheat field at Avon in South Australia during the winter of 1996. The surface of the eggs was examined under the scanning electron microscope and was shown to be highly ornamented and reticulate with numerous "inverted goblet-shaped" projections. The dentate margins of the heads of these projections consist of clusters of coral-like globules. Statistical analysis of the size of the projections revealed two significantly different types of eggs in the samples. Examples of ornamented eggs were found in discarded exuvia, a condition which is not in accord with a recently proposed hypothesis on tardigrade egg evolution. Observations on feeding behaviour, population density and egg laying habits are presented.

KEY WORDS: *Macrobiotus* cf. *pseudohufelandi*, microscopy, tardigrades, eggs, morphology, evolution, soil.

Introduction

Tardigrades, also known as water bears or moss-piglets (Kinchin 1994), belong to a discrete phylum of cosmopolitan distribution from diverse habitats including marine, fresh water and semi-terrestrial environments. The tardigrades responsible for the eggs described in this paper were identified as *Macrobiotus* cf. *pseudohufelandi* Iharos 1966 by S. Claxton (Bird 1996) and are semi-terrestrial, having been isolated from sandy loam soil in a wheat field at Avon, South Australia.

The tardigrade egg shell is a useful taxonomic guide to species identification, particularly in genera such as *Macrobiotus* where the shell is ornamented. *Macrobiotus* cf. *pseudohufelandi*, which are only about 500 µm long by 150 µm wide when fully grown, lay comparatively large circular eggs which have highly ornamented reticulated shell surfaces with numerous "inverted goblet-shaped" projections (Bird 1996).

It has been shown by Bertolani & Rebecchi (1993) that differences in egg shell morphology in *Macrobiotus hufelandi*, previously thought to be due to variability within this species, fall into seven distinct types that are related to different animal morphotypes. Using egg shell morphology, along with other characters, these workers have described a number of new species from the *M. hufelandi* group. Eggs that belong to this group have pitted or reticulated shells with protruding processes shaped like inverted goblets, chalices, thread spools or "cooling towers".

Bertolani *et al.* (1996) have stated that ornamented eggs are generally laid free in soil or water and smooth-shelled eggs are laid in the moulted cuticle (exuvium). These workers include the family Macrobiotidae, to which *M.* cf. *pseudohufelandi* belongs, in those families that lay free, ornamented eggs. Bertolani *et al.* (1996) have put forward an hypothesis in which they explain the evolution of tardigrade eggs.

In this paper we examine the structure of the egg shell of *M.* cf. *pseudohufelandi* and measure the processes protruding from the surface of the egg shell. We also discuss its egg-laying habits in relation to the hypothesis of Bertolani *et al.* (1996) and comment on feeding behaviour and population density.

Materials and Methods

The tardigrades were collected on 18 July 1996 from sandy loam soil and from the same locality at Avon, South Australia (latitude 34° 14′ S, longitude 138° 19′ E), as those collected previously, using the sampling technique described by Bird (1996). The soil samples were collected in mid-winter so that the sites were wet and the tardigrades were feeding, reproducing and depositing eggs.

The tardigrades and other meiofauna, consisting predominantly of nematodes, were isolated from this soil over a period of three days using a misting apparatus (Yeates & Bird 1994). Tardigrades and nematodes were counted and tardigrade eggs were picked out using a dental No. 3 nerve broach and examined, alive, in distilled water under a coverslip, with the light microscope. Eggs to be examined under the scanning electron microscope (SEM) were fixed in

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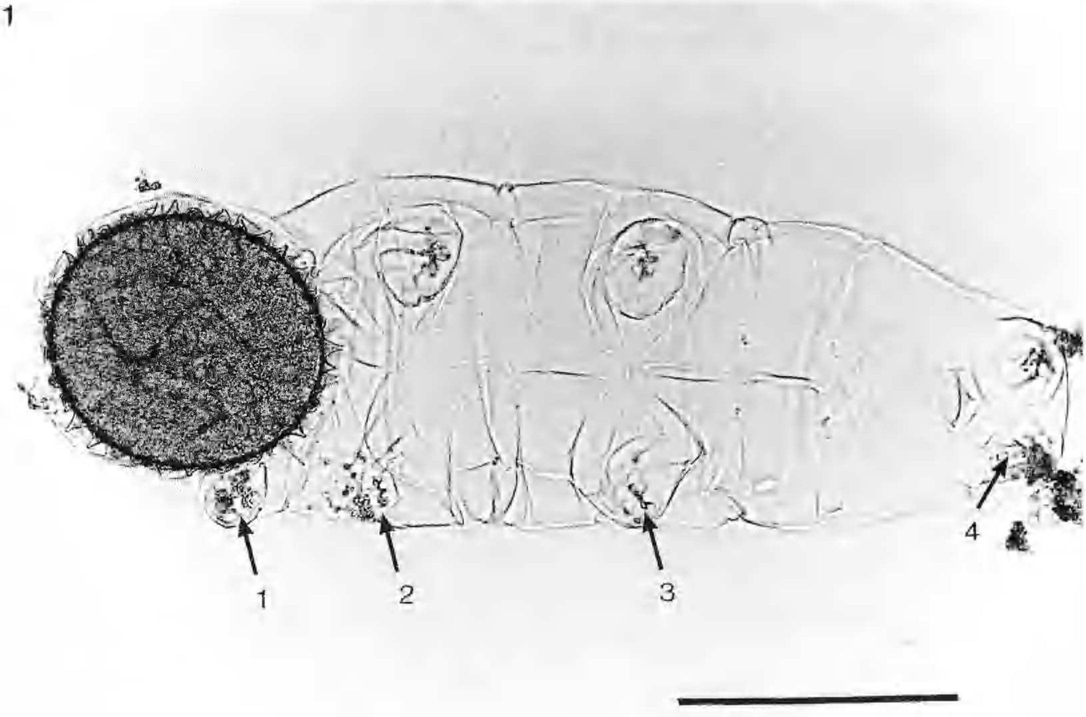

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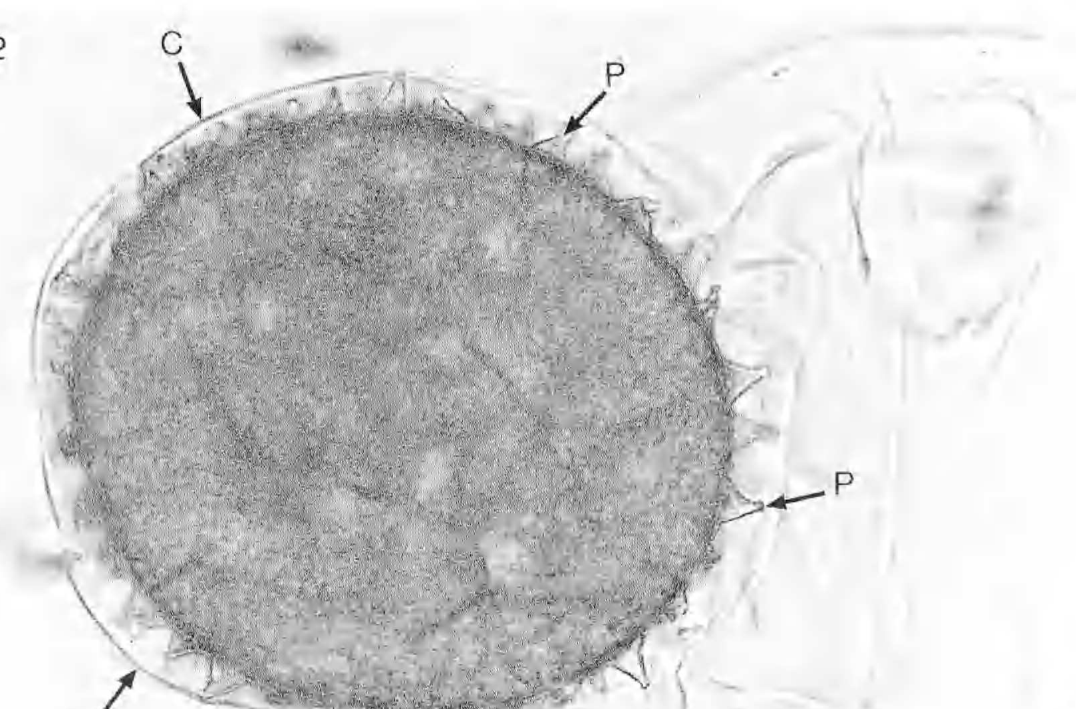


6% gluteraldehyde in Sørensen's phosphate buffer at pH 7.2 and at 5° C and kept in this fixative for several days. They were then washed three times in distilled water and sonicated in an Elma T420 sonicator at a frequency of 35 kHz for 10 sec or until shown by microscopic observation to be free of debris.

The eggs were freeze-dried by placing them between membrane filters which were frozen rapidly by placing them in a slurry of freon cooled by liquid nitrogen. These filters with the eggs were quickly transferred to a freeze drier and freeze-dried at - 70° C. The dried eggs were picked up with double-sided tape which was attached to an SEM stub and coated with 30 nm of gold. This material was examined and photographed in a Cambridge S 250 Mk 3 SEM operated at 20 kV using FP4 Plus Ilford roll film.

Results

Numbers and feeding

At the time of collection the ratio of tardigrades to nematodes in 50 g of soil was 84:297. Feeding on nematodes was also observed during the course of this investigation and the nematodes held by the tardigrades usually did not move although once a nematode broke free and moved away. In one instance, a tardigrade was observed to be arching its back in the manner of a scorpion, during feeding activity.

Egg laying and eggs

Eggs that were about 90-100 μm in diameter in the living unfixed state (Figs 1, 2) and about 60-70 μm in the fixed and dehydrated state (Fig. 3), were laid either within exuvia (cast cuticles) (Figs 1, 2) or free (Fig. 3). Figures 2, 3, 4, 5 clearly show that these eggs have a morphology of the *M. hufelandi* group with reticulated shells and characteristically upturned-chalice-shaped protruding processes. Although the pattern of the reticulations remains the same with the apertures on the reticulate surface of the egg shell being about 0.25 μm in diameter (Fig. 6), the shape of the protruding processes falls into two distinct groups. Type I is shorter than Type II, is narrow at the base and has a wide distal head (Fig. 4). Conversely, Type II is taller, wider at the base and has a narrower distal head than Type I (Fig. 5). For 10 processes of each type, the differences in the means are statistically significant with 95% confidence (Table 1).

When the processes were examined under the higher magnification of the SEM (Fig. 6), the dentate cog-shaped margins of the head were shown to consist of clusters of globules with a structure resembling madreporarian corals in appearance and were approximately 0.5 μm in diameter. The madreporarian globules and reticulated shell surfaces are similar in both the shell types described above.

Discussion

Bertolani *et al.* (1996) have proposed an hypothesis to explain the evolution of tardigrade eggs. According to these workers, the eggs of tardigrades have evolved as a result of two events, the first being the acquisition of ornamentation and the second the use of the shed exuvium as the site for egg laying, with the subsequent loss of ornamentation. Thus, the ornamented eggs of the Macrobiotidae are thought to be laid free. Our observations that the ornamented eggs of *M.* cf. *pseudohufelandi* can either be laid in exuvia (Figs 1, 2) or free (Fig. 3) do not appear to be in accord with this hypothesis. The exuvia containing ornamented eggs were transparent and devoid of body contents and did not appear to be females that had died before completing egg laying. A possible explanation for the laying of the eggs in the exuvium by the *M.* cf. *pseudohufelandi* from the soil at Avon,

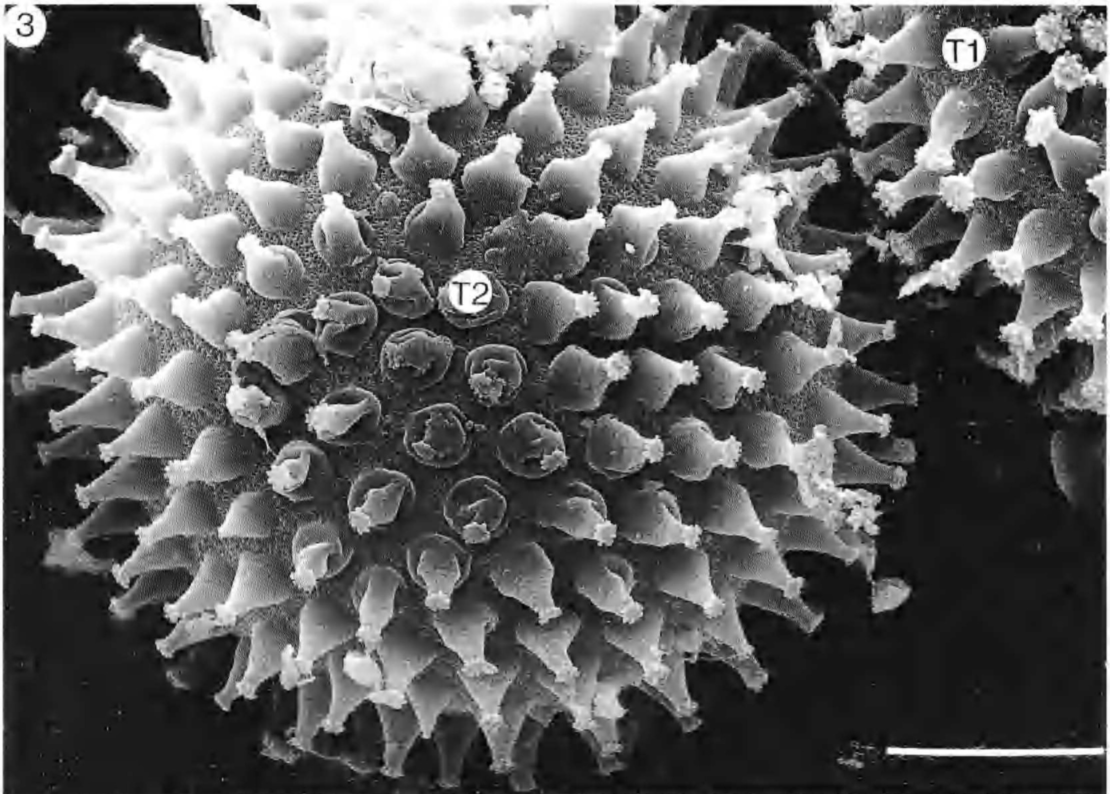
TABLE 1. *Measurements of the two types of processes protruding from the egg shell surfaces of* Macrobiotus *cf.* pseudohufelandi.

Type	No.	Height (μm)			Basal width (μm)			Distal Width (μm)		
		Mean	± SD	Range	Mean	± SD	Range	Mean	± SD	Range
I	10	5.9	0.5	5.4-6.5	4.3	0.2	3.9-4.8	3.3	0.2	3.0-3.7
II	10	6.4	0.4	5.7-7.4	5.3	0.2	4.9-5.6	2.1	0.1	2.1-2.4
t [0.95] > 2.26		2.47			11.18			16.97		

SD = Standard Deviation, t = Student's t Test

Fig. 1. Egg of *Macrobiotus* cf. *pseudohufelandi* in cast cuticle (exuvium). Arrows indicate position of cast legs and claws. Bright field optics. Scale bar = 100 μm.

Fig. 2. Same specimen at higher magnification showing shell projections (P) covered by cast cuticle (C). Bright field optics. Scale bar = 20 μm.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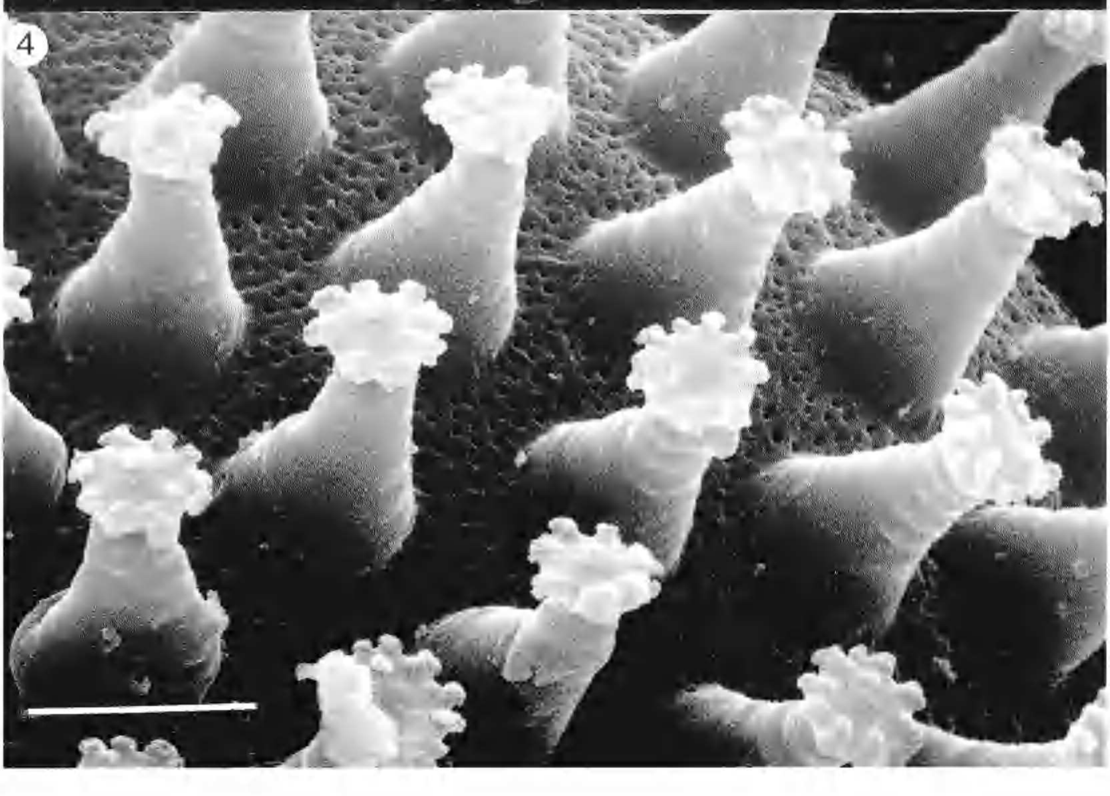
T1
T2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is that this may be an adaptation to the hot, dry summers experienced there.

Our studies on the tardigrades and nematodes in soils from wheat fields at Avon in midwinter when the soils were wet and the populations of the meiofauna could be expected to be at their peak, show that tardigrades make up a substantial component, although they are not as numerous as nematodes. The ratio of 84 tardigrades to 297 nematodes per 50 g soil found in these experiments varies at other sites where the tardigrade numbers per 50 g soil may be less and nematode numbers greater (Bird 1996). However, it is clear that the tardigrade presence at Avon is widespread.

The tardigrades isolated from soil at Avon feed on nematodes and can survive hot dry summers in an anhydrobiotic state (Bird 1996). Feeding on nematodes was also observed during the course of this investigation and the nematodes held by the tardigrades did not usually appear to be moving, although they were coiled and therefore probably not dead, suggesting to us that they might have been paralyzed by some type of injected narcotic. Since there is no information on tardigrade diversity and geographical distribution in South Australia, further studies are warranted, particularly on their feeding habits, as they may have a role in the biocontrol of the parasitic nematodes which have been shown to occur in the wheat fields at Avon (Yeates & Bird 1994).

The fine structure of the egg shell in the genus *Macrobiotus* is of great taxonomic importance. From our observations on the ultrastructure of the egg shells of the Avon tardigrades, it would seem that there may be two populations of *M.* cf. *pseudohufelandi* in the Avon soil or there may be two different species, neither of which completely resembles those described so far for the *hufelandi* group (Bertolani & Rebecchi 1993; Biserov 1996). The reticulated surface of the shell and the structure of the globules on the heads of the projections are similar in the two forms of eggs described above but the diameter of the apertures on the reticulated surface of the egg shell is much less than that shown in the eggs of other members of the *hufelandi* group described by Bertolani & Rebecchi (1993), Kinchin (1994) and Biserov (1996). To our knowledge, the globules on the heads of the projections have not been described before and might prove, together with the reticulated surface, to be useful taxonomic criteria if the *hufelandi* group is further divided on the basis of egg ornamentation.

Clearly further studies are required on the taxonomy of these tardigrades and on their distribution in the semi-arid agricultural areas of South Australia and other similar regions of the Australian continent.

Acknowledgments

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Fig. 3. Scanning electron micrograph of whole eggs of *Macrobiotus* cf. *pseudohufelandi* showing the inverted goblet-shaped projections of Types I (T1) and II (T2). Scale bar = 20 µm.

Fig. 4. Scanning electron micrograph of part of the egg shell surface of a Type I egg showing projections with narrower bases and larger dentate cog-shaped heads than those of Type II. Note similarly reticulated surfaces of egg shells. Scale bar = 5 µm.

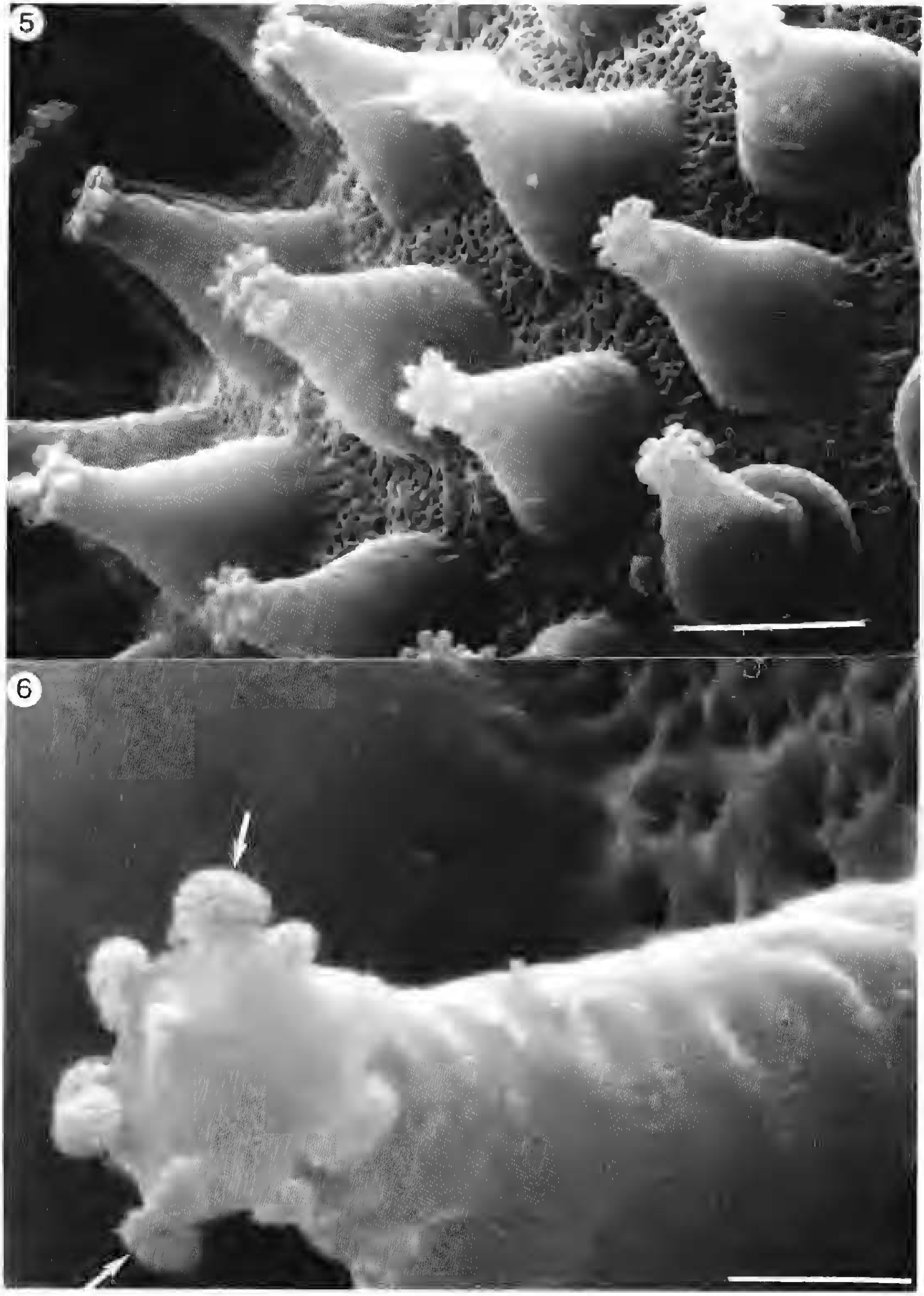
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Fig. 5. Scanning electron micrograph of part of the egg shell surface of a Type II egg showing projections with wider bases and smaller cog-shaped heads than those of Type I. Note similarly reticulated egg shell surfaces. Scale bar = 5 µm.

Fig. 6. Scanning electron micrograph of the head of one of the Type II projections at higher magnification showing the madreporarian globules (arrows). Note the size of the apertures on the reticulated surface of the egg shell on the top right hand side of the photomicrograph. Scale bar = 1 µm.